United States Environmental Protection Agency

Predicting Compound Amenability with Liquid Chromatography Mass Spectrometry to Improve Non-targeted Analysis

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United States Environmental Protection Agency

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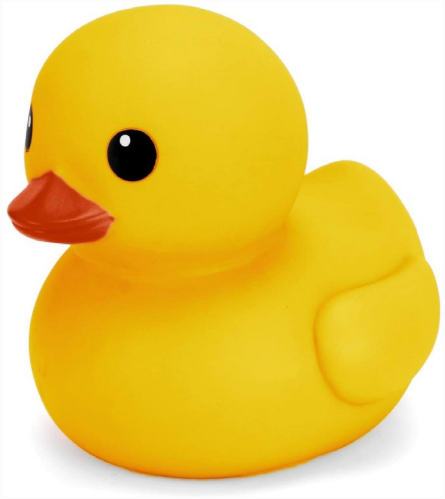
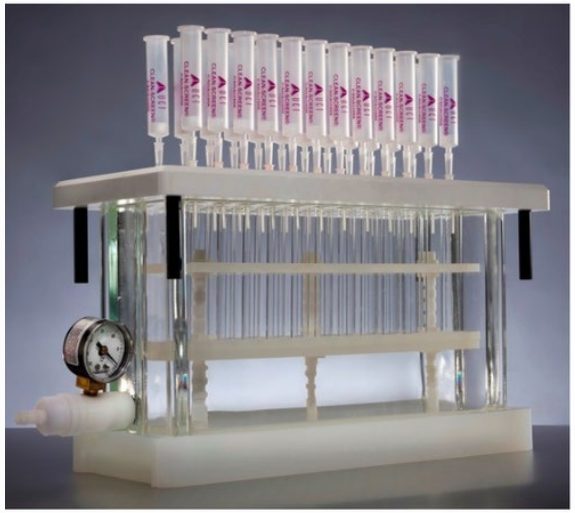
Complex samples, NTA, and the modeling problem

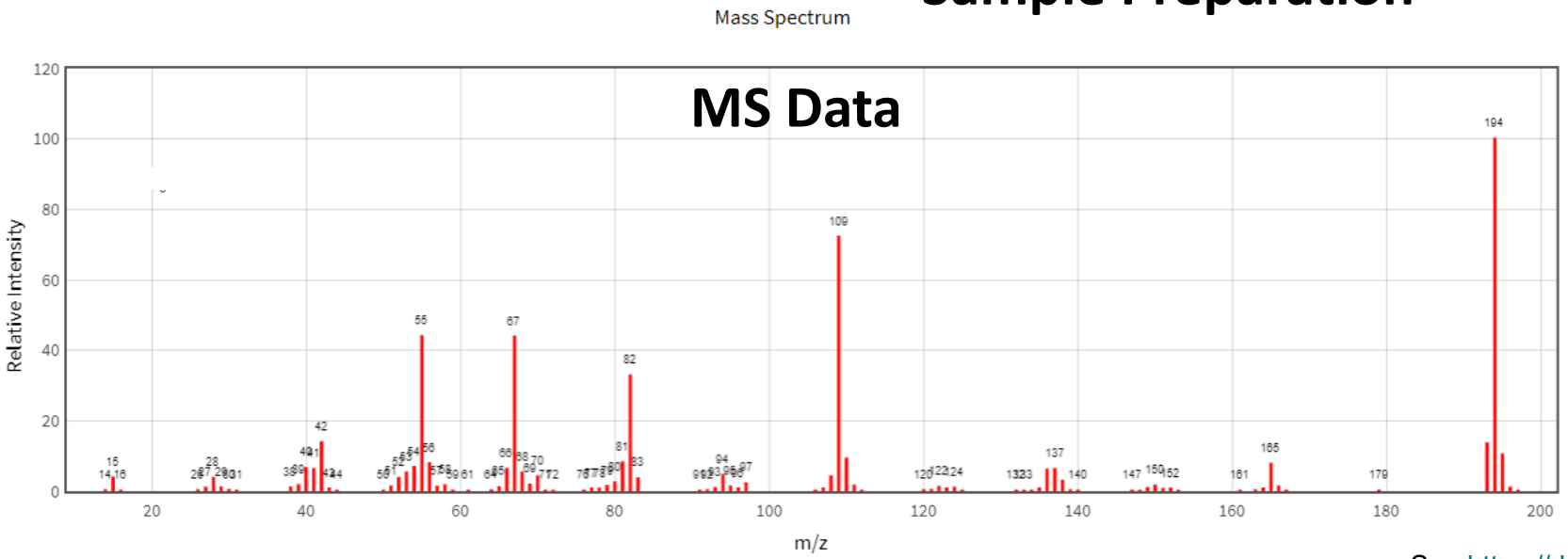


Media Sample



Extraction, Cleanup & Sample Preparation

MS Analysis



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See https://doi.org/10.1021/acs.est.1c01907 for more on rubber ducky analysis.

Curating a dataset for modeling

MassBank of North America

- **4,103 unique compounds detected in ESI+ LC-MS**
- **3,007 unique compounds detected in ESI- LC-MS**
- **1,542 unique compounds detected in both modes**

- Only amenable compounds identified in MoNA
  - No unamenable compound data
- ToxCast library LC-MS/MS curation
  - Spectra checked individually for quality
    - Provides unamenable compound data

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Agilent

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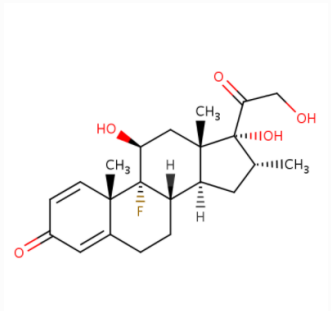
- **ESI+ LC-MS/MS**
  - **393 amenable; 456 unamenable**
- **ESI- LC-MS/MS**
  - **456 amenable; 402 unamenable**

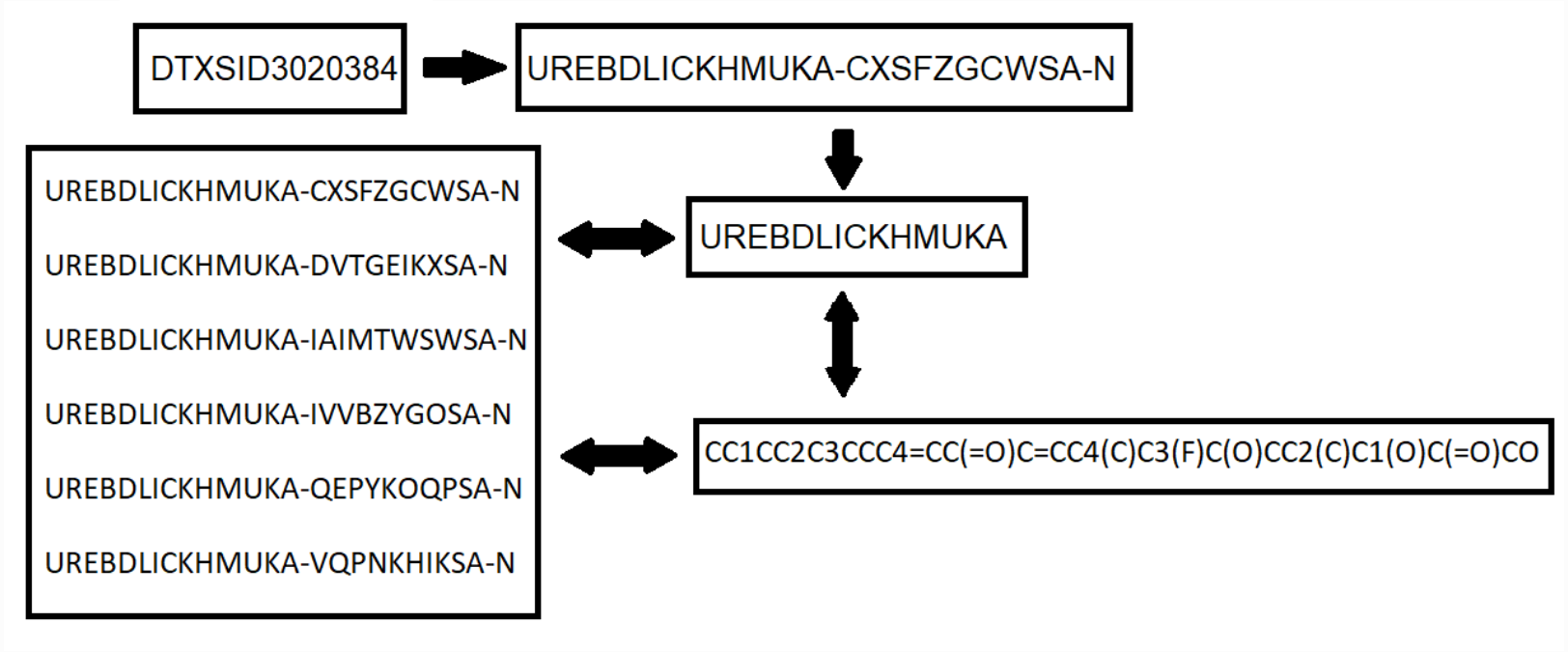
Curating a dataset for modeling

Overall dataset

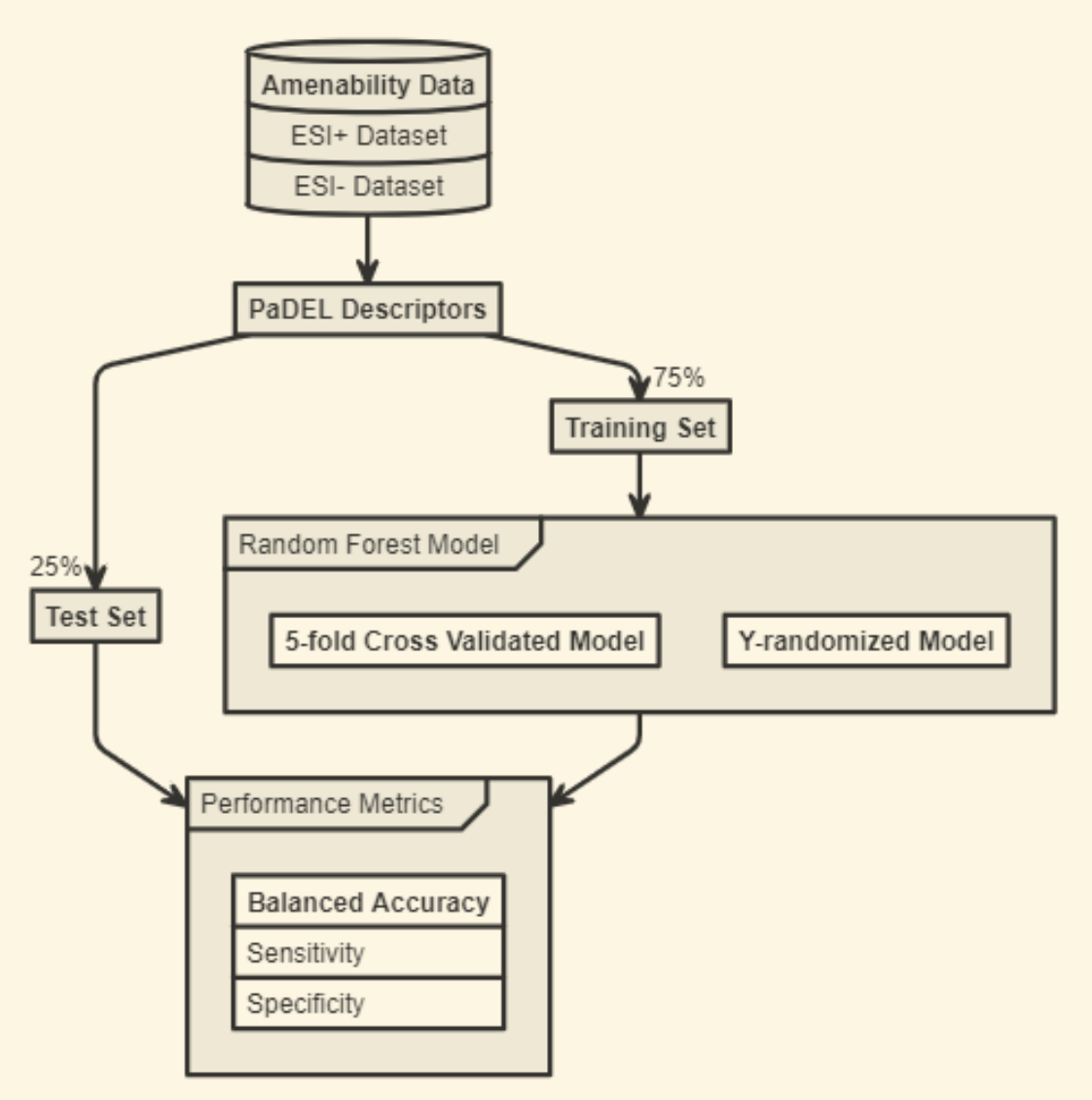
- **ESI+ LC-MS/MS**
  - **4,226 amenable; 387 unamenable**
- **ESI- LC-MS/MS**
  - **3,130 amenable; 360 unamenable**

Curating a dataset for modeling





Machine learning approach



Performance Metrics

$$Sensitivity = \frac{TP}{TP + FN}$$

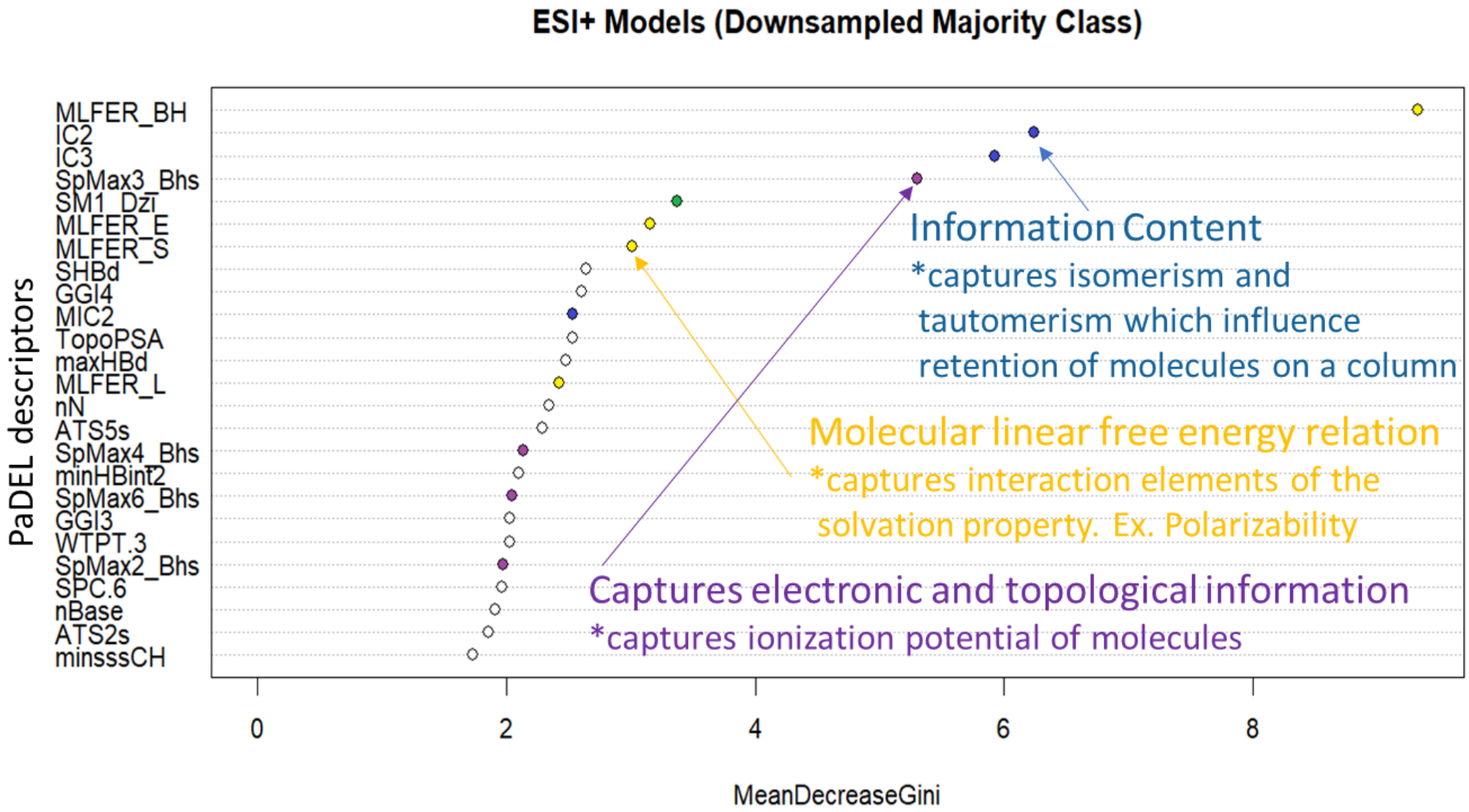
$$Specificity = \frac{TN}{TN + FP}$$

$$\text{balanced accuracy} = \frac{\text{sensitivity} + \text{specificity}}{2}$$

Model performance

	Training Set				Fivefold CV		
Model	Size	Balanced Accuracy	Sensitivity	Specificity	Balanced Accuracy	Sensitivity	Specificity
ESI+ Models (Downsampling Applied)	580	0.78	0.79	0.77	0.77	0.76	0.78
ESI+ Models (Upsampling Applied)	6340	0.99	1.00	0.99	0.99	0.98	1.00
ESI- Models (Downsampling Applied)	550	0.83	0.82	0.84	0.81	0.83	0.79
ESI- Models (Upsampling Applied)	4688	0.99	1.00	0.98	0.98	0.97	1.00
	Test Set				**Y-randomization**		
Model	Size	Balanced Accuracy	Sensitivity	Specificity	Balanced Accuracy	Sensitivity	Specificity
ESI+ Models (Downsampling Applied)	1153	0.81	0.85	0.76	0.48	0.44	0.51
ESI+ Models (Upsampling Applied)	1153	0.58	0.98	0.19	0.55	0.48	0.63
ESI- Models (Downsampling Applied)	871	0.82	0.85	0.80	0.50	0.49	0.51
ESI- Models (Upsampling Applied)	871	0.68	0.99	0.38	0.51	0.46	0.56

Mechanistic interpretation

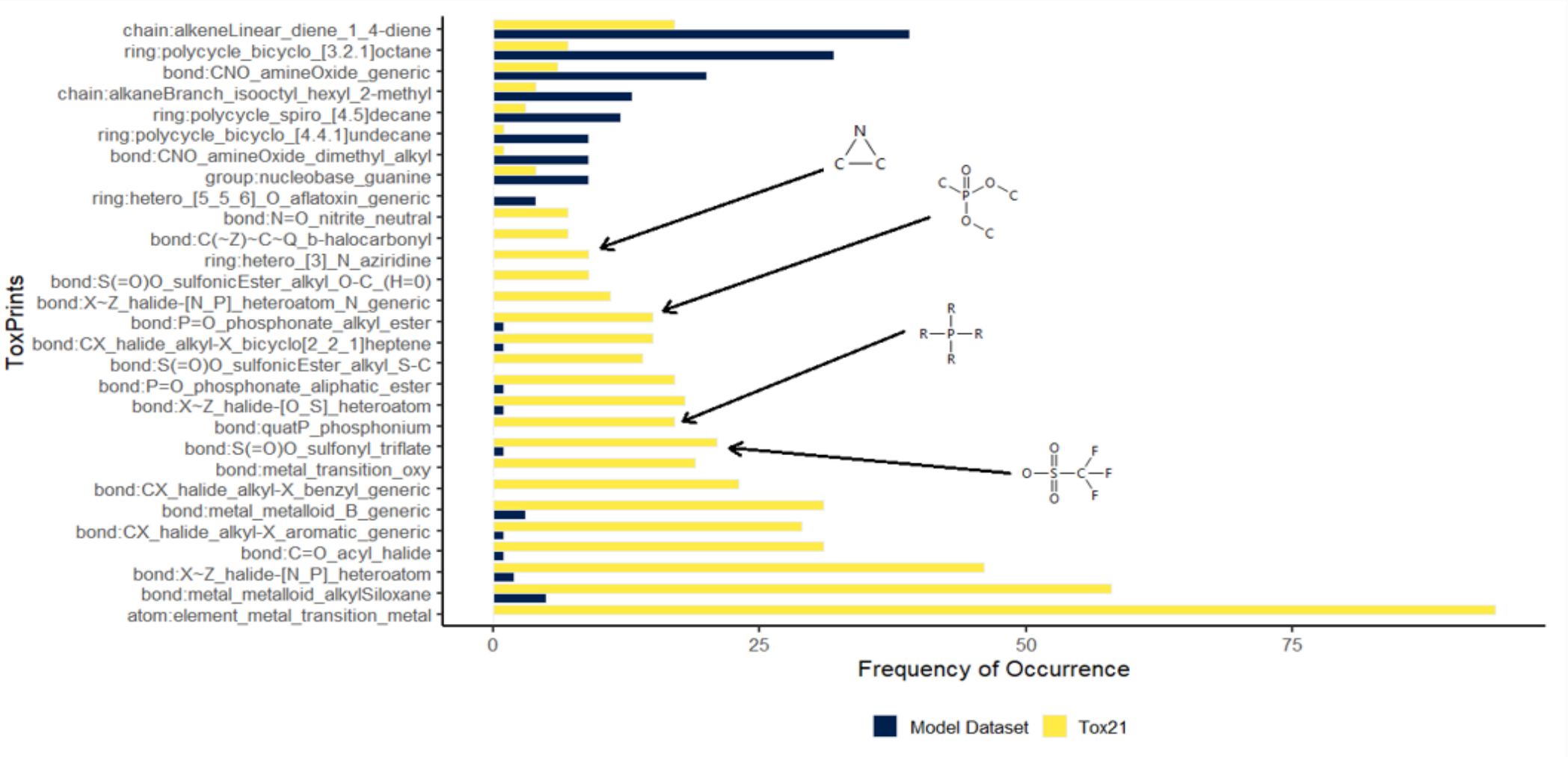


External validation

- 1,768 chemical compounds analyzed for LC-MS amenability as part of ToxCast program
  - All compounds exclusive of modeling dataset

ESI- Downsampled Model		
	Amenable (Prediction)	Unamenable (Prediction)
Detected (Experiment)	323	502
Not-detected (Experiment)	68	874
Sensitivity	**0.83**	
Specificity	**0.64**	
Balanced Accuracy	**0.73**	
ESI+ Downsampled Model		
	Amenable (Prediction)	Unamenable (Prediction)
Detected (Experiment)	423	402
Not-detected (Experiment)	103	839
Sensitivity	**0.80**	
Specificity	**0.68**	
Balanced Accuracy	**0.74**	
Combined Models		
	Amenable (Prediction)	Unamenable (Prediction)
Detected (Experiment)	505	320
Not-detected (Experiment)	129	813
Sensitivity	**0.80**	
Specificity	**0.72**	
Balanced Accuracy	**0.76**	

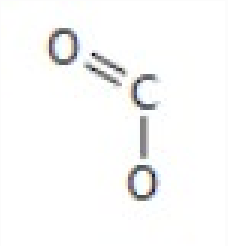
Model applicability to ToxCast



Comparison of prevalent ToxPrint chemotypes in amenability dataset against the ToxCast dataset

Model comparison with expert intuition

- A small molecule containing a carboxylic acid functional group *should* be amenable to ESI- LC-MS

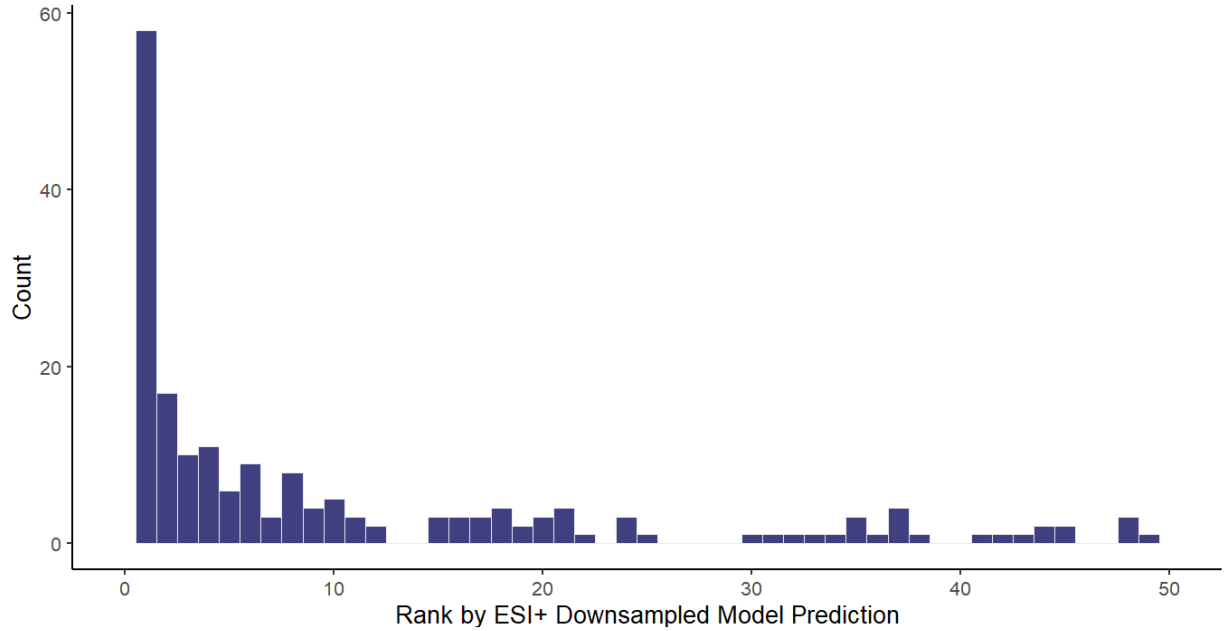


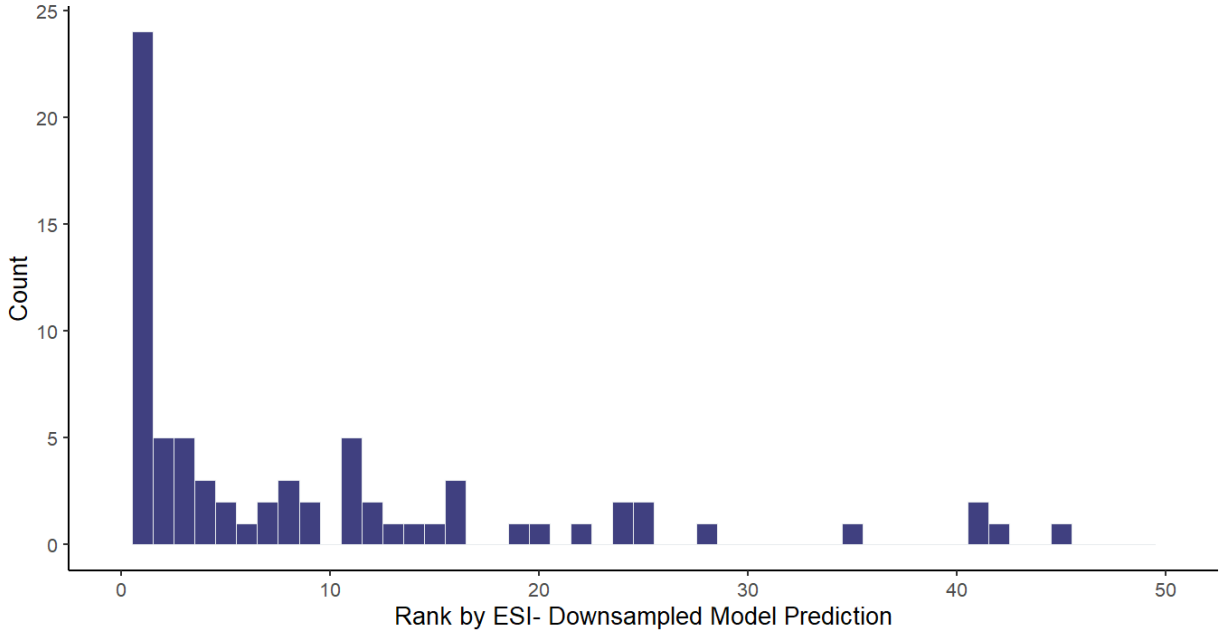
- 773 compounds contained the ToxPrint “bond:C(=O)O_carboxylicAcid_generic” in amenability dataset

ESI- Downsampled Model		
	Amenable (Prediction)	Unamenable (Prediction)
Detected (Experiment)	728	4
Not-detected (Experiment)	37	9
Sensitivity	**0.95**	
Specificity	**0.69**	
Balanced Accuracy	**0.82**	

Suspect-screening application

- List of ENTACT compounds identified in ESI+ & ESI- LC-MS
  - 228 in ESI+
  - 108 in ESI-
- Retrieved candidates for each molecular formula via Dashboard
  - 13,325 candidates for ESI+
  - 7,079 candidates for ESI-
- Generated amenability predictions for candidate structures
- Rank ordered candidates by amenability probability (probability of detection)





Summary

- Amenability models are available as a deployable API inside of a Docker container
- These models will be used in the ChemSpace tool currently in early development
  - Please check out our ChemSpace poster!

Research Paper | Published: 14 October 2021

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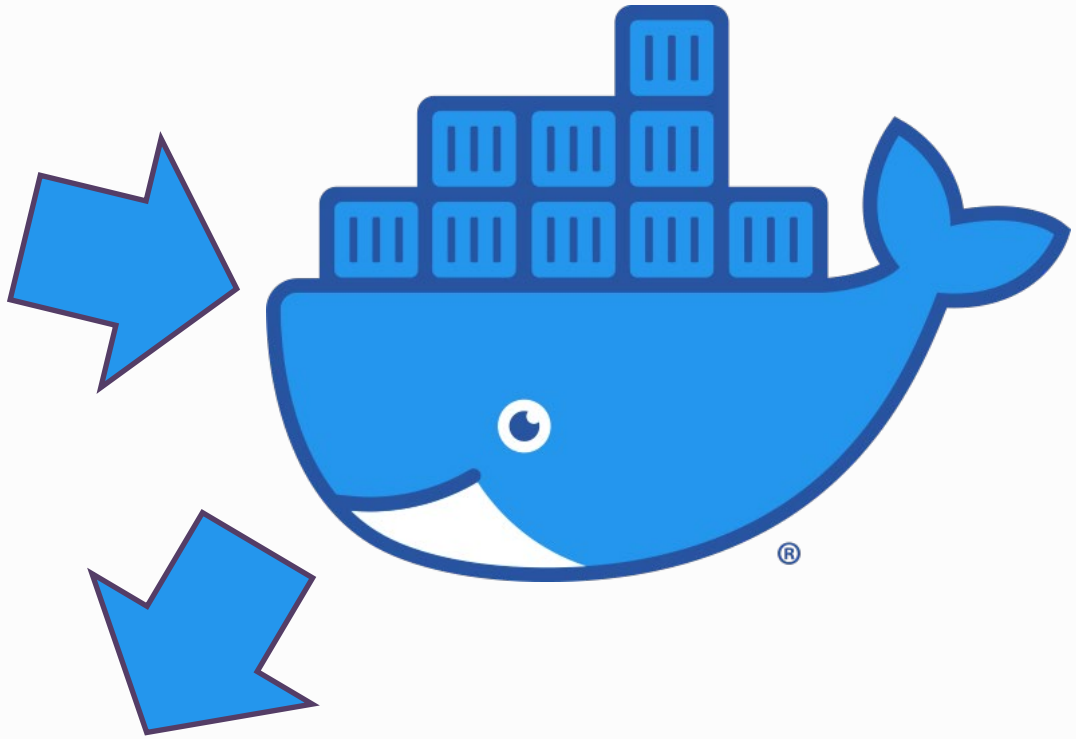

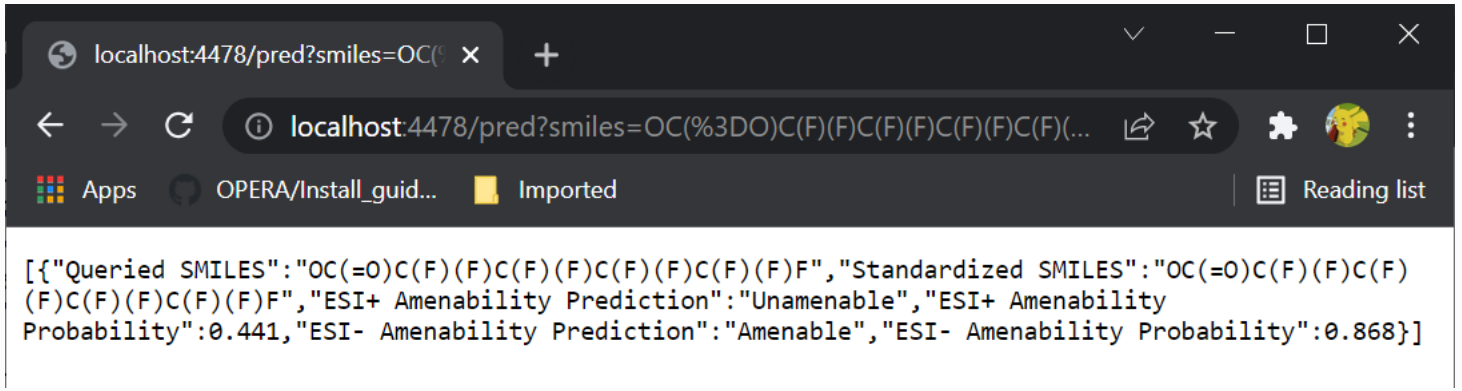

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Thank you for
Listening!